Patent Abstracts of Japan

PUBLICATION NUMBER : 07059812
PUBLICATION DATE : 07-03-95

APPLICATION DATE : 27-08-93
APPLICATION NUMBER : 05213096

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INT.CL. : A61F 13/00 A61L 15/16

TITLE : WOUND COVER MATERIAL AND ITS PRODUCTION

ABSTRACT : PURPOSE: To obtain the wound cover material which facilitates the exudation of the leaching liquid out of the inside of the body particularly on a wound surface, has an enhanced adhesion property and exhibits an early recovery as the wound cover material designed to treat a burn, external injury, wound, etc.

CONSTITUTION: This wound cover material consists of a collagen sponge structural body having a so-called honeycomb structure which has the cell diameters controlled to a 50 to 2000μm range and is formed with the cells communicating straight from one surface to the other surface and the cells substantially independent from each other.

Kokai (Japanese Unexamined Patent Publication) No. 7-59812
Title of the Invention: Wound Cover Material and Its Production method Publication Date: March 7, 1995
Patent Application No. 5-213096
Filing Date: August 27, 1993
Applicant: Koken Co., Ltd.

[ABSTRACT]

[Purpose] The object of the present invention is to provide a wound cover for the treatment of burns, external injuries and so forth that enhances adhesion by facilitating the exudation of exudate from inside the body particularly on a wound surface to exhibit rapid healing.

[Constitution] The present invention relates to a wound cover material comprised of a collagen sponge structural body having a so-called honeycomb structure that has air bubbles controlled to a diameter within the range of 50-2000 μm, said air bubbles communicating straight from one surface to the other surface, and each of said air bubbles being substantially independent from each other.

[SCOPE OF CLAIM FOR PATENT]

[Claim 1] A wound cover material comprised of a collagen sponge structural body having a so-called honeycomb structure that has air bubbles controlled to a diameter within the range of 50-2000 μm, said air bubbles communicating straight from one surface to the other surface, and each of said air bubbles being substantially independent from each other.

[Claim 2] A wound cover material comprised of a collagen sponge structural body having a so-called honeycomb structure that has air bubbles controlled to a diameter within the range of 50-2000 μm, said air bubbles communicating straight from one surface to the other surface, and each of said air bubbles being

substantially independent from each other; wherein, cells are incorporated into said collagen sponge structural body.

[Claim 3] A production method of a wound cover material having air bubbles controlled to a diameter within the range of 50-2000 μm, said air bubbles communicating straight from one surface to the other surface, and each of said air bubbles being substantially independent from each other, comprising: forming a gelatinous body in which straight water columns are formed from one surface to the other surface by exposing an acidic solution of collagen to ammonia gas, and subsequently volatilizing the moisture inside the gel by freeze-drying.

[DETAILED DESCRIPTION OF THE INVENTION]

[Industrial Application Field]

The present invention relates to a wound cover material, and more particularly, to a wound cover material that enhances adhesion to a wound surface by facilitating the exudation of exudate from within the body particularly on a wound surface to exhibit rapid healing.

[Prior Art]

Wound cover materials of various types of materials and forms have been developed and used practically in recent years. Materials can be divided into two types consisting of synthetic materials and natural materials, while examples of forms include sheets, sponges and non-woven fabrics. Among these, application research has been conducted particularly actively on collagen in consideration of the superior bioaffinity of its extracellular matrix. The use of collagen as a wound cover material in the form of a sponge is also known, and example of this can be found in Japanese Examined Patent Publication No. 61-41452. However, in the case of simply freeze-drying a collagen solution for the method of producing a collagen sponge body, independent air bubbles are only formed randomly, thereby making it difficult to obtain a form that is suitable for use

as a wound cover material. In addition, in the case of recently developed wound cover materials having cells, namely wound cover materials used for the purpose of promoting healing by various physiologically active factors produced by cells, it is difficult to obtain a form that is desirable with respect to the objective of adhering cells to the inside or surface of the cover material and allowing those cells to propagate so that the factors produced by those cells demonstrate effects on the wound surface.

[Problems to be Solved by the Invention]

As a result of the inventors of the present invention improving on the shortcomings of a previous production method for a collagen sponge structural body of the prior art, and conducting various studies to obtain a cell culture collagen support that enables superior cell adhesion, propagation and enhanced culture density, they found that when a collagen solution is brought near the isoelectric point of collagen by ammonia gas, simultaneous to the collagen being neutralized, moisture is separated in the form of cylindrical water columns from one surface to the other surface within collagen gel, and by then freeze-drying this, an extremely porous collagen sponge can be produced having air bubbles substantially communicating in a straight line from one surface to the other surface, and that this collagen sponge is superior as a cell culture collagen support, thereby leading to the invention of a cell culture collagen support and its production method (see Japanese Unexamined Patent Publication No. 4-204239).

As a result of additional studies, the inventors of the present invention also found that the collagen sponge body having the above characteristics is has extremely superior effects as a wound cover material, thereby leading to completion of the present invention. Thus, the object of the present invention is to provide a wound cover material that enhances adhesion to a wound surface by facilitating the exudation of exudate from inside the body on a wound surface to exhibit rapid healing.

[Means for Solving the Problems]

The gist of the present invention is a wound cover material comprised of a collagen sponge structural body having a so-called honeycomb structure that has air bubbles controlled to a diameter within the range of 50-2000 μm, said air bubbles communicating straight from one surface to the other surface, and each of said air bubbles being substantially independent from each other; the above wound cover material in which cells are incorporated in the collagen sponge structural body having the above characteristics; and a production method of a wound cover material having a so-called honeycomb structure in which the diameter of air bubbles is controlled within the range of 50-2000 μm comprising forming a gelatinous body in which straight water columns are formed from one surface to the other surface simultaneous to neutralization of collagen by exposing an acidic solution of collagen to ammonia gas, and subsequently volatilizing the moisture inside the gel by freeze-drying.

Namely, the present invention is a wound cover material in which each air bubble, controlled so that its diameter is within the range of 50-2000 μm, is substantially independent, and communicate from one surface to the other surface, the elimination of exudate is difficult in the case the diameter is less than 50 μm, cells cannot be cultured as a result of not being able to enter inside, and in the case the diameter exceeds 2000 μm, the carrier becomes excessively large making culturing efficiency poor, and the walls become thin making it impossible to obtain strength, thereby making this undesirable. A collagen sponge having such an air bubble diameter is superior as a wound cover material whether it is used directly as a wound cover material or in a state in which it is incorporated with cells.

In the production method of the present invention, a collagen solution is brought close to the isoelectric point of collagen by exposing to ammonia gas, the collagen is neutralized to a

gel, the moisture is separated into cylindrical water columns in the collagen, and by freeze-drying this moisture, a collagen sponge is formed that has air bubbles present in a straight line from one surface (top surface layer) to the other surface (back surface layer) that are also substantially independent of each other. In this production method, the diameter of the air bubbles can be controlled by regulating the amount of collagen and concentration of the ammonia gas, thereby making it possible to change the pore size of the collagen sponge.

The following provides a more detailed explanation of the present invention. The collagen used in the present invention may be any collagen provided it is soluble in acid and forms collagen fibers in the presence of ammonia gas, preferable examples of which include atherocollagen and acid-soluble collagen. This collagen is dissolved in an acidic solution. Examples of acidic solvents that may be used include inorganic acids such as hydrochloric acid and acetic acid, and organic acids, and there are no particular restrictions on the pH of their solutions. Although there are also no particular restrictions on the concentration of collagen, it is preferably about 0.1-10%. When this collagen acidic solution is exposed to ammonia gas, the collagen precipitates into fibers extending straight from one surface to the other surface, the solution becomes turbid, and the moisture separates into water columns.

At this time, the ammonia gas is either introduced into a sealed container containing the collagen solution from a gas tank, aqueous ammonia for which concentration has been adjusted is placed inside a sealed container containing collagen, to neutralize. During this neutralization, the diameter of the air bubbles can be adjusted by adjusting the concentration of collagen and the concentration of ammonia gas. For example, the concentration of ammonia gas in the sealed container should be at least 100 ppm in order to neutralize a solution having a collagen concentration of 1%. In addition, in the case of

a wound cover material that is expected to promote healing due to the effects of physiologically active factors produced by cells incorporated therein, skin fibroblasts, epidermal cells or their combination and so forth can be used as incorporated cells.

Since this cover material is able to allow the adhesion and propagation of these cells over the entire surface of the air bubble walls, cells can be cultured to a high density. In addition, since the air bubbles are arranged in a single direction, namely in the form of honeycombs, the supply of nutritive capabilities and the release of product is facilitated for the cells, and the resulting cover material has superior properties as a wound cover material. Cells following growth subculturing of the patient's own cells or cells from another person can be used for the cells. In addition, at the time of their use, the cells may be adhered and propagated prior to use or freeze-dried following propagation, and then thawed at the time of use. Regardless of the manner in which they are used, the thickness of the cover material is preferably within the range of 0.5-20 mm.

In addition, the cover material can be used after introducing crosslinking for the purpose of minimizing degradation following freeze-drying. Examples of crosslinking methods include physical crosslinking such as heat, UV irradiation or γ-rays, chemical crosslinking using formalin, glutaraldehyde, hexamethylene diisocyanate or a polyepoxy compound, or any combination thereof. In addition, the present wound cover material can be used by producing after inserting an antibiotic and so forth inside. In the case of using the present wound cover material, it is used extremely easily at the time of use as a result of being observed to exhibit satisfactory adhesive properties and hemostatic effects. The following provides a detailed explanation of the present invention using its embodiments.

[Embodiments]

Embodiment 1

After dividing a 1.0% atherocollagen (pH 3.0) solution into 300 g aliquots in 10 x 20 cm trays, two of the above trays were placed in a sealed container having a volume of 5 liters. 30 ml of 3.0% aqueous ammonia in a 50 ml container were additionally placed in this container and allowed to stand for 12 hours at room temperature. Following standing, after washing the formed gel overnight with running water, freeze-drying was performed to obtain sponge having a pore size of 300-500 μm. Each of the air bubbles were substantially independent, and were communicating from one surface to the other surface. This was then sliced to a thickness of about 2 mm and the surface layer was irradiated with UV light (wavelength in the vicinity of 255 nm) for 20 minutes at a time at an intensity of 500 $\mu W/cm^2$ followed by sterilization and use as a wound cover material. Namely, the wound cover material of the resulting collagen sponge was applied to a wound in which all skin layers were missing on the back of a rat. The area of missing skin measuring 1 cm x 1 cm was created surgically, the collagen sponge was placed over the wound and fixed in place by covering with gauze. Observations were made 5, 10 and 15 days later, and efficacy was confirmed by examining for hemostasis, adhesion and reconstruction of the dermis and epidermis as determined by histological examination. In addition, a healthy, reconstructed surface was obtained following healing.

Embodiment 2

During the ammonia neutralization of Embodiment 1, 500 g aliquots of collagen solution were placed in each tray, while neutralization, washing and freeze-drying were carried out under similar conditions to obtain a sponge having a pore size of 800-1000 μm. Each of the air bubbles was substantially independent, and communicating from one surface to the other

surface. This was then sliced to a thickness of about 2 mm and the surface layer was irradiated with UV light (wavelength in the vicinity of 255 nm) for 20 minutes at a time at an intensity of 500 $\mu W/cm^2$ followed by sterilization and use as a wound cover material.

Embodiment 3

During the ammonia neutralization of Embodiment 1, 150 g aliquots of collagen solution were placed in each tray, while neutralization, washing and freeze-drying were carried out under similar conditions to obtain a sponge having a pore size of 100-400 μm. Each of the air bubbles was substantially independent, and communicating from one surface to the other surface. This was then sliced to a thickness of about 2 mm and the surface layer was irradiated with UV light (wavelength in the vicinity of 255 nm) for 20 minutes at a time at an intensity of 500 $\mu W/cm^2$ followed by sterilization and use as a wound cover material.

Embodiment 4

After dividing a 1.0% atherocollagen (pH 3.0) solution into 300 g aliquots in 10 x 20 cm trays, two of the above trays were placed in a sealed container having a volume of 5 liters. 30 ml of 3.0% aqueous ammonia in a 50 ml container were additionally placed in this container and allowed to stand for 12 hours at room temperature. Following standing, after washing the formed gel overnight with running water, freeze-drying was performed to obtain sponge having a pore size of 300-500 μm. Each of the air bubbles of this sponge body were substantially independent, and were communicating from one surface to the other surface. This was then sliced to a thickness of about 2 mm, placed in a methanol solution containing 0.05% hexamethylene diisocyanate and allowed to react for 6 hours at room temperature followed by thoroughly washing the sponge with methanol and drying to obtain a wound cover material.

Embodiment 5

After dividing a 1.0% atherocollagen (pH 3.0) solution into 300 g aliquots in 10 x 20 cm trays, two of the above trays were placed in a sealed container having a volume of 5 liters. 30 ml of 3.0% aqueous ammonia in a 50 ml container were additionally placed in this container and allowed to stand for 12 hours at room temperature. Following standing, after washing the formed gel overnight with running water, freeze-drying was performed to obtain sponge having a pore size of 300-500 μm. Each of the air bubbles of this sponge body were substantially independent, and were communicating from one surface to the other surface. After slicing this to a thickness of about 2 mm, 150 mg of sponge subjected to UV irradiation and sterilization treatment in the same manner as Embodiment 1 were placed in a medium (DME + 10% FBS) to remove the air bubbles followed by dispersal and adhesion of 1 x 10^6 human fibroblasts and culturing for 5 days in a CO_2 incubator to obtain a wound cover material.

Embodiment 6

After dividing a 1.0% atherocollagen (pH 3.0) solution into 300 g aliquots in 10 x 20 cm trays, two of the above trays were placed in a sealed container having a volume of 5 liters. 30 ml of 3.0% aqueous ammonia in a 50 ml container were additionally placed in this container and allowed to stand for 12 hours at room temperature. Following standing, after washing the formed gel overnight with running water, freeze-drying was performed to obtain sponge having a pore size of 300-500 μm. Each of the air bubbles of this sponge body were substantially independent, and were communicating from one surface to the other surface. After slicing this to a thickness of about 2 mm, UV irradiation and sterilization treatment were carried out in the same manner as Embodiment 1, and after additionally culturing human fibroblasts according to the method described in Embodiment 5, the entire collagen film of human epidermal cells cultured on the collagen film was placed on the sponge to obtain a wound cover material.

[Effect of the Invention]

As has been described above, in the present invention, since air bubble diameter can be controlled by the concentration of ammonia gas, in addition to being able to obtain a honeycomb-shaped collagen cover material having an air bubble diameter suitable for use as a wound cover material, a cover material can also be obtained that is suitable when promoting wound healing by incorporating cells. In addition, a healthy healed surface can be obtained when this cover material is used.